

Establishing Tolerable Dungeness Crab (*Cancer magister*) and Razor Clam (*Siliqua patula*) Domoic Acid Contaminant Levels

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ABSTRACT

Domoic acid has been found in razor clams (*Siliqua patula*) and dungeness crab (*Cancer magister*) in Washington State and elsewhere on the west coast of the United States. Due to toxic effects associated with domoic acid exposure, an effort has been made to establish tolerable domoic acid levels in crab and clam obtained from commercial harvest and sale and from individual recreational harvesting. To accomplish this: 1) the amount of clams and crabs consumed by populations of concern was determined, 2) a tolerable daily intake (TDI) was developed for individuals most sensitive to effects of this compound, and 3) the TDI was equated with consumption patterns to determine tolerable clam and crab domoic acid levels. Results indicate that the primary health effects associated with domoic acid toxicity can be averted in populations of concern and for others consuming crab or clams less frequently (or in lesser quantity) if domoic acid contaminant concentration does not exceed 30 mg/kg in the hepatopancreas and viscera of dungeness crab or 20 mg/kg in clam.

INTRODUCTION

In 1987, more than 100 individuals became ill after consuming cultured mussels (*Mytilus edulis*) harvested off the Canadian Province of Prince Edward Island (1, 2). The mussels were contaminated with the potent neuroexcitatory amino acid domoic acid (domoate), a toxin not previously observed in shellfish (1, 3). Produced by a diatom (*Pseudo-nitzschia multiseries*), domoic acid acted as an acute toxicant to cause illness and subsequent death in three elderly individuals and to cause permanent short-term memory loss in several survivors of the 1987 incident (4, 5). Vomiting, abdominal cramps and diarrhea were observed within the first twenty four hours of exposure (1, 5). After two days, the more severely affected individuals showed confusion, disorientation and other neurological effects.

On the west coast of the United States, domoic acid has recently been found in razor clams (*Siliqua patula*) and dungeness crabs (*Cancer magister*) (6, 7). Clams are consumed in substantial quantity when recreational harvesting is permissible. Of all clam body parts, the foot (digger) contains the highest levels of domoic acid and is considered a delicacy by some individuals (6). Individuals who regularly harvest clams recreationally comprise the population of greatest concern since these individuals usually eat the harvested clams.

Crab viscera (entrails) contain nearly all of the toxicant found in crabs, with the hepatopancreas (crab “butter” or “mustard”) having the greatest proportion (6, 7). Cooking practices impact exposure to domoic acid since cooking crab in boiling water lowers total toxicant content by reducing viscera concentrations significantly (8). As a result, individual exposure would be highest when crab are prepared using a method other than boiling (such as steaming or frying) and when viscera are consumed with the meat. One must determine consumption rates of crab and crab viscera (including the hepatopancreas) to determine if populations are at risk from domoic acid toxicity. Along with recreational clam harvesters, individuals of ethnic Chinese descent are considered to be a highly exposed population since many of them consume whole crab. Approximately 34,000 individuals of direct Chinese ancestry live in the state of Washington, with nearly 26,000 residing in the greater Seattle area (9).

To protect these and other populations, crab and clams obtained for consumption must have domoic acid concentrations that will not cause adverse effects. The goal of this study is to determine such concentrations of domoic acid. This paper addresses the public health implications of exposure to domoic acid in crabs and clams by: 1) acquiring data on the amount of clam or crab viscera consumed (along with other information pertaining to crab and clam consumption) from two separate and distinct consumption pattern surveys conducted in Washington State, 2) a tolerable daily intake (TDI) for individuals most sensitive to the effects of domoate was derived using available toxicity data, and 3) the consumption data results were equated with the TDI to determine tolerable clam and crab domoic acid levels that would protect public health by not producing the deleterious effects associated with domoic acid toxicity in individuals consuming these seafoods.

METHODS

CONSUMPTION SURVEYS

Separate and distinct consumption pattern surveys were conducted during 1993 and 1994 in Washington. This was done to acquire data on the amount of clam and crab consumed by the population recreationally harvesting clams and by the Chinese community. A pilot survey was included to aid in questionnaire design. All persons responsible for surveying the individuals were trained prior to study initiation.

Pilot Survey

A pilot survey using a clam digger questionnaire was performed on five recreational harvesters to determine what modifications were necessary. A pilot survey using a restaurant questionnaire was performed in two restaurants to determine what, if any, changes were required so that all essential data could be gathered. A pilot survey using the questionnaire designed for the individuals surveyed (restaurant owners, chefs, waitpersons, newspaper respondents, and University of Washington students/scholars) was performed on more than ten Chinese individuals who ate crab to ascertain if the questionnaire was clear and understandable and to decide what alterations were required.

Survey Information Statement

Before each survey was conducted the interviewer provided written and oral information (in English, Chinese or one of its dialects) to the individual being surveyed stating that the survey was voluntary and he or she was free to not answer any question.

Clam Survey

On the last day of recreational clam harvesting season in March of 1993, interviewers conducted surveys on the coast of Washington near Ocean Shores. Interviewers were dropped off at known recreational harvesting locations along the beach. Interviewers conducted interviews during the same time period so that no one individual would be surveyed twice. To aid the respondent during the survey, the respondent was shown a large laminated pictorial of a razor clam with all pertinent parts labeled. This was done so that both parties had the same reference point when discussing which portions of the clam were or were not consumed. The questionnaire addressed the amount consumed, repeat exposure, methods of cooking, and frequency of consumption. If children were present, the accompanying adults were asked about the child's consumption patterns.

Crab Survey Samples

Several methods were used to interview a broad spectrum of the Chinese community in Washington so that various subgroups were represented. Four surveys were implemented to render data from numerous sections of the Chinese community which, when combined, provided for a large cross-section of the population. The restaurant survey was initially conducted as a method of gaining insight into food consumption patterns of people of ethnic Chinese descent without relying on interviews conducted in the home. Door-to-door in-home interviews were not

conducted in the Chinese community because of the difficulty in properly defining the Chinese population so that a randomized sample from all different socio-economic strata could be selected, and because the approaches required to determine ethnicity (i.e., screening) would possibly be considered as impolite or discourteous questioning in this culture (Z. Ye, University of Washington, Seattle, WA, personal communication).

The individuals surveyed, consisting of restaurant owners, chefs, waitpersons, newspaper respondents, and University of Washington students/scholars allowed for access to population subgroups throughout the community. The survey of owners/cooks/waitpersons was to identify if there were differences between how the meals were prepared in the restaurant and at home.

Crab Survey: Restaurants

Approximately thirty owners of authentic Chinese restaurants that serve crab were approached, of which twenty agreed to participate in the crab survey. To be eligible, restaurants had to serve crab on a regular basis (daily during season) or occasionally (at least once a week). Two of the twenty restaurants included in the survey results fell into the latter group. The restaurants were usually family-owned with the owners directly involved in daily affairs such as cooking, cleaning, accounting, and waiting on tables. Individuals knowledgeable in meal preparation at the restaurant were interviewed in Chinese and were provided with \$10 upon completion of the survey. The questionnaire dealt with cooking practices, items served containing crab (such as entrees, soups, and sauces), quantity of crab used, number of individuals served per entree, and popularity of served items. If verbal descriptions for each crab portion were insufficient, the interviewer used laminated enlarged color photographs of dissected crab to provide detailed views of the gills, stomach, hepatopancreas, remaining viscera, and meat.

Crab Survey: Restaurant Cook/ Owner/ Waitperson

An individual who was knowledgeable of the cooking practices in the restaurant and in their home was surveyed at each of the twenty restaurants. The purpose of this interview was to determine similarities between restaurant and home cooking practices. Since salads are often eaten at home, questions pertaining to the use of crabs in salads were included.

Crab Survey: Internet/ E-mail

Since many students use the Internet for communication and for information gathering, a survey was conducted using this medium as a method of accessing a section of the Chinese community. The solicitation and questionnaire on crab consumption patterns were sent to the individual responsible for network-management for the University of Washington Associated Chinese Students and Scholars. Subsequently, both items were sent twice over a period of two weeks to each member of the user's group. The solicitation and questionnaire indicated that the survey could be completed and returned to a specific address via e-mail. All who completed the questionnaire would be reimbursed \$2 for their efforts.

Crab Survey: Newspaper Advertisement

A week-long advertisement was placed in the World Journal (Daily News) newspaper. It is a Chinese newspaper published by World Journal Incorporated of California, with various editions released daily for different sections of the country. The Seattle edition is for the Northwest and has a circulation of approximately 5,000 in greater Seattle. The first day of advertising was done

in the Friday paper since that day's paper has the largest circulation. Included in this newspaper, along with the advertisement, was an article discussing the project and its purpose. The advertisement and article indicated that a survey on crab consumption was being conducted and that \$2.00 would be presented to anyone who participated in the survey. The reader was provided with a local telephone number and was requested to place a call to the interviewer so that the survey could be conducted.

Crab Survey Verification and Clarification

For each of the surveys conducted, at least ten percent of participants were contacted a second time to ascertain if the questionnaire was clear and understandable and to repeat certain questions to clarify any discrepancies that may have existed. For each survey that indicated consumption of gills and stomachs, the individual (or restaurant) was contacted for clarification and verification, since the stomach, which includes the cardiac stomach, contains a lateral tooth that makes this organ inedible. Also, gills are usually not consumed since many toxins are stored in this organ (Z. Ye, University of Washington, Seattle, WA, personal communication).

TOLERABLE DAILY INTAKE

A Tolerable Daily Intake (TDI) was developed for this study based on a review of epidemiological and toxicological literature. Special attention was given to studies which identified effects at low level exposures; i.e., studies that provided information on sensitive toxic endpoints. The following is a brief overview of several studies which guided the selection of a TDI. This section also represents a portion of the domoate review conducted for this study.

Human Data

In the 1987 outbreak connected with contaminated mussels, one case (that showed effects) was documented for every 500 exposed individuals who did not show effects. Of the 107 that met the case definition for mussel-associated intoxication, nearly half were between 40 and 59 years of age with 36 percent 60 years or older (5). Younger individuals were more likely to have diarrhea, whereas men and older adults were more likely to have memory loss and to require hospitalization (5). In explaining the sex difference, Perl et al. (1990) suggest that only the most severely affected men consulted physicians or contacted health departments. Also of note was that all severely ill individuals less than 65 years-old had pre-existing illnesses, with poor renal function being the common predisposing factor. Poor renal function may also explain the differences between younger individuals and older adults, since renal function can decrease with age, thereby increasing the half-life of domoic acid in the body.

Truelove and Iverson (1994) (10) exposed cynomolgus monkeys (*M. fascicularis*) to domoic acid (i.v.); within six hours all primates had eliminated half of the original dose via urine. Similar pharmacokinetic results have been reported by Scallet et al. (1995) (11). If this half-life is similar to that of humans, repeat exposure (i.e., day to day) should not produce any increase in body burden. In older adults and individuals (both young persons and older adults) with a compromised renal function due to illness, the half-life of domoic acid could increase sufficiently so that repeated exposure over 12 or 24 hrs may be significant.

With respect to older adults, longitudinal studies have shown that the rate of glomerular filtration, estimated by determining clearance of inulin, urea, or creatinine, declines with age

after maturity (12 - 15). Although this decrease does occur with age in many individuals, a more recent longitudinal study on the rate of decline in renal function with age conducted by Lindeman and co-workers (1985) (16) suggests that age adjustments made for renal function based on mean population data can have substantial error when applied to specific individuals. In the population studied, one third of all subjects had no absolute decrease in renal function and a small group had statistically significant increase in function with age, even though there was an observed mean decrease in creatinine clearance with age (0.75 ml/min/year). Clinically, age-adjusted modifications for renal function decrease are made based on a creatinine or inulin clearance of 1 ml/min/yr after age 40 years. This adjustment is made particularly for drugs excreted by the kidneys and equates to an individual 80 years-old having renal capacity approximately 60% of that in a young person. Although this provides for a better estimate than if no correction were made, the most accurate method for determining renal function is on an individual basis (16). Although there may be an undetected pathology in a portion of this "normal" population (individuals on diuretics and antihypertensives and those with possible renal or urinary tract disease were removed), a mean creatinine decrease was evident in most of the population. This decrease from a progressive involutional process occurring with normal aging will make this population more sensitive since the excretion time of domoic acid via the kidney may be lengthened. Also, these findings suggest that in a population that has compromised renal function beyond the progressive involutional process observed with normal aging, the half-life of domoic acid could be even further increased. Presently, there is insufficient evidence to quantify this decrease in renal function for either older adults or for those with existing renal impairment. However, this evidence does suggest that there is a sensitive population, of which many are older adults, that deserves attention when establishing tolerable domoic acid intake levels.

Domoic acid exposure levels for those consuming mussels during the 1987 outbreak were not generally known. However, for ten older adults, nine of whom were described as cases, it was possible to determine mussel consumption along with actual domoic acid levels in remaining mussels not consumed (5). These limited data showed a relationship between domoic acid exposure and observed symptoms. The one individual having no symptomatology consumed 0.2 - 0.3 mg domoic acid/kg body weight. Mild symptoms (e.g., gastrointestinal distress, nausea) were observed in individuals (n = 7) exposed to 0.9 - 2.0 mg/kg (5).

Animal Data

Rodent models were important in establishing domoic acid as a toxin of concern and have been beneficial in defining the neurotoxic impact of domoic acid exposure. However, rodent data will not be discussed in any great detail since experimental results, including observed clinical responses, have been dissimilar to the toxin exposure-response relationship observed in humans. This dissimilarity may result from the rat's inability to vomit, since similar doses (i.v. and i.p.) cause hippocampal damage in rats and monkeys (17 - 19).

Experiments involving cynomolgus monkeys have provided great insight to the dose-response relationship of domoic acid as well as to neurological damage produced from toxin exposure. In humans, memory loss was observed in several cases. Memory is associated with the CA1 and CA3 regions of the hippocampus and the mediodorsal nucleus of the thalamus (20). These three areas were damaged in autopsied human brain tissue from the 1987 incident (21). Cynomolgus monkeys exposed to domoic acid (about 0.5 mg/kg i.v.) showed damage to mossy fiber terminals of the hippocampal region (CA2) which contains the greatest kainic acid receptor concentration in the brain (17). Kainic acid receptors are involved with glutamate transmission, and domoic acid, a potent agonist of this receptor, has a greater affinity for the receptor than does kainic acid

(22-25). Binding of domoic acid to the receptors is thought to be a critical step in domoic acid induced neurotoxicity (17, 22, 23). The monkeys also showed damage to the pyramidal neurons and axon terminals in the subfields CA1, CA2, CA3, CA4 and presubicular regions of the hippocampus at somewhat higher doses (about 1 mg/kg i.v.). An approximately four-fold greater sensitivity to domoate-induced neurotoxicity was observed in adult animals as compared to juveniles. These results are in agreement with previous work indicating that mussel extract and purified domoate damaged the CA1, CA3 & CA4 subfields of the hippocampus (17, 26, 27).

The similarity of neurological damage (particularly on hippocampal subfields) produced by domoic acid in both humans and monkeys suggests that the monkey model can provide great insight into effects seen in humans at various exposure levels, including levels less toxic. In single oral dose experiments with monkeys, an immediate similarity was noted between clinical signs observed in humans and in these non-human primates (28). No effects were observed at an oral dose of 0.5 mg/kg, but typical human reactions (e.g. mastication, vomiting) were observed at a dose an order of magnitude greater. Additional studies using oral doses of 0.5 mg/kg to 1.0 mg/kg indicate that the 1.0 mg/kg dose initiated a clinical response whereas doses of 0.5 mg/kg and 0.75 mg/kg did not produce any symptoms (29).

Primate studies have exclusively used purified domoate, assuming perhaps that a slight increase of domoic acid in the hippocampus may be responsible for observed neurotoxic effects, despite a blood-brain barrier which limits kainic acid as well as domoic acid access to the brain (30, 31). Other excitotoxins, however, may also be involved in producing these neurotoxic effects. Rodent studies compared extracts of contaminated mussels (containing domoate) with pure domoic acid and with extracts of non-contaminated mussels spiked with domoate (25). Results indicate that the extract of contaminated mussels was the most potent formulation. Based on a series of reproducible behavioral changes that were dose-dependent, dose-response curves for these three formulations produced TD₅₀ values that clearly indicated that contaminated extracts were significantly more toxic than the other two formulations (25). Furthermore, studies using cultured rat cerebellar neurons also suggest that contaminated mussel extract is more neurotoxic than purified domoic acid (32). This increase in neurotoxicity for cultured neurons was due to domoic acid potentiation of the excitatory effect produced by glutamate and aspartate; both are excitatory amino acids (EAAs) found in mussel extracts. Although these experiments indicate the possible synergistic effect of these EAAs in the presence of domoic acid, quantifying this effect for application to humans or primates is presently not possible although it may prove to be of importance to the human situation. Further work with animal models will be required to determine if aspartic and glutamic acid get to the target organ (hippocampus) in sufficient quantities to elicit increased neurotoxicity, and if target organ concentrations exceed concentrations obtained from a normal diet.

Establishing the TDI

To establish a TDI for domoic acid, human and primate data are required. Since domoate can produce effects from a single exposure, acute low-level exposure data are used until experimental neurotoxic evidence becomes available to suggest the relevance of chronic low-level exposure. Human data indicate that older adults, individuals with renal dysfunction, and possibly men comprise the most sensitive population. No data is available to provide insight into effects on these sensitive individuals after low-level exposure. Only one individual from the 1987 outbreak is known to have consumed domoic acid in this low-level range (0.2 - 03 mg/kg) and that individual had no symptomatology. As a result, any TDI established for domoate must include a safety factor (33, 34) to protect this sensitive population. Additionally, renal function can impact

the half-life of domoic acid, and until this impact is properly understood and quantified, a cautious approach using a safety factor to protect certain sensitive individuals is warranted.

Evidence indicates that humans and cynomolgus monkeys have similar dose-response relationships for various endpoints; therefore the usual safety factor (33, 34) applied for intraspecies variation would not be required. Neurological effects produced from domoate exposure are similar in humans and primates, with damage occurring in hippocampal subfields. Non-human primate oral toxicity studies indicate that effects were not observed at the 0.75 mg/kg and 0.5 mg/kg domoic acid exposure level, but clinical effects (i.e. vomiting) were present in primates exposed to 1.0 mg/kg. For individuals where it was possible to determine mussel consumption along with actual domoic acid levels in mussels consumed, a 1 mg/kg exposure level resulted in gastrointestinal effects.

Based on the above data, a value of 0.75 mg/kg (lowered an order of magnitude to control for sensitive populations) provides sound basis for a TDI. The resulting tolerable daily intake is 0.075 mg/kg.

RESULTS

Clam Survey

A total of 135 questionnaires were filled out by trained interviewers. Almost all respondents were Caucasian, and divided equally by gender. Almost two of every three individuals interviewed were considered by the interviewer to be over 50 years in age. The number of children present was insufficient to determine their consumption patterns. When preparing clams, greater than 90% of the respondents (n=129) fry them; 75% use them to prepare soup or chowder. No other preparation category (i.e., raw, canned, baked or boiled) was identified by more than 3% of the respondents. In the category of "clam parts not eaten", only one of 130 respondents to that question acknowledged that the clam was eaten as a whole. Of the remaining 129, more than 75% of the individuals removed the necktip, the gills and entrails before consuming the clams.

Of respondents answering the question "when did you last catch clams?" (n=131), nearly 80% had been digging for clams within the last week, and almost half of those within the last 1 or 2 days. More than two-thirds of those interviewed had harvested the limit of 15 clams the last time they had been digging. Many of these clams may have been frozen for later consumption and/or given to family and friends, since only 37% of those responding (n=120) indicated that they ate clams once or twice a week and 60% indicated that they ate clams less than once a week. Only 3% indicated that they ate clams more than twice a week. When asked if clams were ever eaten two days in a row, 44% indicated that they did so on occasion.

When clams are consumed, sizable portions are eaten at one sitting. Of 125 individuals, nearly 75% ate from three to six clams during a meal (Table 1).

Data obtained during March of that year from clams collected several miles north of the survey area indicate that only 7% of the clams had a length of less than four inches, with the average size being 4.75 inches (D. Molenaar, Quinalt Indian Reservation, Taholah, WA, personal communication.). This size equates to approximately four clams per pound (with shell), of which about 40% can be recovered (for clams caught in the month of March)(D. Molenaar, Quinalt

Indian Reservation, Taholah, WA, personal communication; B. Weidman, Quinalt Tribal Enterprises, Taholah, WA, personal communication). The recovered portion is the clam without necktip, gills, entrails and shell. Thus, one clam has an average edible weight of approximately 45 grams (1.6 oz). For the 75% of respondents who consume between three and six clams at one sitting, an estimated 135 to 270 gms of clams (5 - 10 oz) are eaten.

Crab Surveys

A total of 99 interviews were completed from four samples implemented in this portion of the study. When statistically feasible (35), the data have been pooled so that study samples obtained through various routes into the community can reflect the entire diverse population. Of the 99 surveys completed, 79 came from individuals and the remaining twenty represented restaurants. From pooled data of 79 individuals, 44% occasionally consume crabs on two consecutive days. Frequency of crab consumption was determined for each group (Table 2). Results of pooled data indicate that nearly half of the individuals (48%) consume crab between two and four times per month.

All respondents reported eating crab meat; however, few individuals consumed gills or stomachs (Table 3). After pooling data from all samples, including restaurants, more than half of the respondents indicated that they consumed the hepatopancreas (55%). We were unable to pool samples on consumption of remaining viscera; however, the range of persons consuming this portion of the crab was 30 - 73%.

Restaurants served much less crab per individual than did groups consisting of individual responses ($p < 0.05$) (Table 4). Pooled samples (excluding restaurant data) indicated that, on average, 1.04 crabs were consumed per individual per serving. Nearly half of the individuals (47%) in the pooled samples consume one crab per serving. When divided by amount consumed (pooled samples only), 30% of respondents consumed half a crab or less, 52% consumed from 0.6 crab to 1.0 crab, and only 18% consumed more than one crab per serving.

Respondents to the cook/waitperson/owner surveys indicated that they prepared meals in a similar manner at home as at the restaurant. Crab was not used in salads at home and entrees were the only meal items prepared with crab by the restaurants, with the exception of one restaurant. Since the crab is live until ordered in all restaurants surveyed, use of crab for entrees only is not a surprising result. Results of meal preparation methods showed that boiling crab was not a popular method of preparation (Table 5). Based on pooled samples, the percentage of persons steaming crabs was approximately 80%. The range of respondents frying crabs ranged from 32 - 100% (the samples could not be pooled). The Student and Scholar Association respondents did not fry the clams but preferentially steamed them.

Tolerable Clam - Domoic Acid Level

A razor clam-domoate level (TL) can be determined as follows:

$$TL = \frac{TDI \cdot W_{oa}}{CL_{0.84}}$$

where:

TDI = tolerable daily intake (0.075 mg/kg),

W_{oa} = average body weight for an older adult (70.0 kg),

CL = razor clam consumption level for portion of the population (0.270 kg for 84th percentile).

The equation provides for a tolerable domoic acid level in razor clams of 19.4 mg/kg (ppm). A variable pertaining to the loss of domoic acid due to cooking was not included since frying, which is the predominant method of cooking, would not significantly reduce domoic acid exposure. A consumption level of 270 gms (6 clams) was used, which represents the 84th consumption percentile (i.e., 6 clams or fewer are eaten by 84% of individuals sampled).

Tolerable Crab - Domoic Acid Level

As with clams, a tolerable crab-domoic acid level can be determined using crab consumption levels. The consumption rate chosen is one crab per individual per serving, which represents the 82nd consumption percentile. Consumed portions of crab consist of meat, hepatopancreas and remaining viscera. Remaining viscera are included since data indicate that they are eaten by the surveyed population. As a result, the tolerable crab-domoic acid level is based on consumption of hepatopancreas and remaining viscera but not meat because meat contains little domoic acid. From measurements of crab taken near British Columbia in 1992, average weight for the combined hepatopancreas and remaining crab viscera portions is approximately 114.0 ± 26.4 gms (R. Chiang, Dept. of Fisheries and Oceans, Burnaby, B.C., Canada, personal communication.). The second deviation from the mean (95th percentile) is used to represent the combined weight portion of crab since there is much variation in weight within the species. Furthermore, high crab viscera weight totals must be included to properly protect public health since a single exposure can produce deleterious effects. The 95th percentile of combined crab weight portion (166.8 gms) is used with the consumption rate (one crab/individual) and the remaining parameters in the above equation to obtain a tolerable domoic acid level in the hepatopancreas and the remaining viscera of dungeness crab equal to 31.5 ppm. A variable pertaining to the loss of domoic acid from cooking is not included since this population prepares crab primarily by steaming and frying, which does not significantly reduce crab-domoic acid content.

For all processed (pre-cooked in water or eviscerated) crab, the meat (leg and body) is not of concern since it retains only about 5 and 10 % (respectively) of the original visceral domoic acid content (8). Of the domoic acid originally in the viscera, 67-71% dissipated with cooking in water. As a result, whole cooked crab will contain less than half of the domoic acid present in a crab eaten by this study's population of concern.

Processed crab are cooked shortly after harvest, whereas crab sold live can be kept between 48 hours and two weeks post-harvest before being consumed (R. Goche, Aquatic Resources Inc., Coos Bay, Oregon, personal communication). During this time period, the crab is starved resulting in the hepatopancreas diminishing in size and concomitantly, domoic acid concentrations decreasing (J. Lund, National Marine Fisheries Service, Seattle, WA, personal communication). Although as much as 60% of the initial domoic acid concentration can dissipate by three weeks post-harvest, a parameter for this decrease was not included in the algorithm determining tolerable crab-domoic acid levels. The variable was omitted since crab consumed within the first several days post-harvest will have little or no decrease in hepatopancreal domoic acid concentrations.

DISCUSSION

This study was initiated to: 1) determine the amount of clams and crabs consumed by populations of concern, 2) establish a TDI for individuals most sensitive to effects of this compound, and 3) derive tolerable clam and crab domoic acid levels that would protect against domoic acid toxicity in individuals consuming these seafoods. Our conclusions will help to avoid future incidents similar to that observed in 1987 after individuals consumed contaminated mussels harvested off the Canadian province of Prince Edward Island. This study provides data on clam and crab consumption from the populations of greatest concern and uses the data with an established TDI to derive domoic acid clam and crab contaminant levels that protect public health.

Results from the clam consumption portion of the study suggest that a high clam consuming population exists and that many (specifically older adults) are part of the sensitive population. Although individuals remove some portions of the clam prior to consumption, everyone eats the foot, where the majority of domoic acid resides. Several clams are eaten at one time, with three out of four individuals consuming 3 - 6 clams during a meal. Repeat exposure to domoic acid may occur from clams eaten by individuals who consume crab on two consecutive days. In Washington, studies indicate that multiple clams in a particular area can be contaminated with domoic acid (6, 7). Therefore, individuals who harvest clams in a single area could collect multiple clams containing domoic acid should the toxin be present. This could effect the TDI once sufficient information is available pertaining to the retention time of domoic acid in individuals within the sensitive population.

Four surveys addressing crab consumption were implemented within the Chinese community to represent various subgroups of the population. The study was designed so that results could be based on data from a large cross-section of the population. Results indicate that a population exists that eats crab, including the hepatopancreas which can contain large amounts of domoic acid. Given that crabs are consumed by nearly half of individuals at least every two weeks and are occasionally consumed on consecutive days, there is ample opportunity for exposure if the crabs were contaminated. As with clams, data on the prevalence of domoic acid in crabs is warranted since this will indicate the chance of consuming contaminated crabs on two consecutive days. While clams can have a high prevalence of contamination, crabs obtained from identical locations in Oregon and Washington have not shown uniformity in domoic acid concentrations (7). As a result, the likelihood of contamination on two consecutive days is significantly less than with clams.

Much information on consumption patterns has been gathered from the two populations in this study; however, further work with increased sample size or with the addition of other populations, such as Native Americans or Koreans, would reinforce and add to our results.

The TDI (0.075 mg/kg-day) was developed acknowledging that older adults, individuals with renal dysfunction, and possibly men comprise the most sensitive population. To establish the TDI, a safety factor was used. This factor was used as a best estimate and was not based on actual data. The safety factor should be replaced once physiologically-based pharmacokinetic data are available to allow quantification of the effect of domoate on older adults or those with impaired renal function. The need for biologically-based, quantitative assessment procedures for neurotoxicants is being addressed (36) and is paramount because this would enable us to better protect public health through the use of scientifically-defined approaches that do not require reliance on uncertainty values.

The TDI and consumption data were used to establish two tolerable crab and clam domoic acid levels that would protect these populations as well as those who consume crab and/or clam less frequently or in lesser quantity. For clams, the level is 19.4 ppm, whereas for crab viscera (hepatopancreas and remaining viscera), the level is 31.5 ppm. Tolerable clam and crab domoic acid levels of 20 ppm and 30 ppm, respectively, should be considered as contaminant levels that will properly protect the public from primary health effects associated with domoic acid toxicity.

The approach presented allows for the review of available toxicity data and the development of a toxicity evaluation that is protective and considers multiple public health concerns. This study has provided information on clam and crab consumption patterns by populations deemed to be high consumers of domoic acid-contaminated species (and their portions). Conclusions suggest that there are distinct, sizable and sensitive populations that may be exposed to domoic acid if clams or crabs become contaminated. Prevalence data for domoic acid must continue to be gathered to assure that tolerable crab and clam domoic acid levels are not exceeded. If data are not collected, deleterious effects could ensue from consumption of clams and crabs containing domoic acid concentrations in excess of 20 ppm and 30 ppm, respectively.

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Table 1. Survey results for clam consumption amount per meal:

		Clams per Meal				
	n (respondents to section)	1-2	3-4	5-6	7-8	9-10
Adults	125	13 10%	51 41%	41 33%	16 13%	4 3%

Table 2. Survey results for crab consumption frequencies in applicable groups:

	n	2-4/mo	1/mo	<1/mo
Cooks/Owners/ Waitpersons	20	5 of 20 25%	11 of 20 55%	4 of 20 20%
Newspaper Respondents	22	13 of 22 59%	8 of 22 36%	1 of 22 5%
Internet E-mail Respondents	37	20 of 37 54%	9 of 37 24%	8 of 37 22%

Table 3. Survey results for crab portions consumed in all groups:

	n	Crab Meat	Gills	Stomach	Hepato- pancreas	Remaining Viscera
Cooks/Owners/ Waitpersons	20	20 of 20 100%	0 of 20 0%	0 of 20 0%	8 of 20 40%	9 of 20 45%
Newspaper Respondents	22	22 of 22 100%	2 of 22 9%	0 of 22 0%	16 of 22 73%	16 of 22 73%
Internet E-mail Respondents	37	37 of 37 100%	2 of 37 5%	0 of 37 0%	22 of 37 60%	11 of 37 30%
Restaurants	20(19) ^a	19 of 19 100%	1 of 19 5%	1 of 19 5%	8 of 19 42%	13 of 19 69%

^a One restaurant owner declined to answer which portions were used.

Table 4. Survey results for quantity of crab consumed in all groups:

	n	Avg. # of Crabs/Individual /Serving	Variance (Crabs/Individual)
Cooks/Owners/ Waitpersons	20	0.93	0.47
Newspaper Respondents	22	0.80	0.79
Internet E-mail Respondents	37	1.25	1.05
Restaurants	20(19) ^a	0.33	0.03

^a One restaurant owner declined to answer the question pertaining to the number of individuals served.

Table 5. Survey results for meal preparation method for crab in all groups:

	n	Fried^a	Steamed^b	Boiled^c
Cooks/Owners/ Waitpersons	20	19 of 20 ^d 95%	13 of 20 ^d 65%	0 of 20 ^d 0%
Newspaper Respondents	22	10 of 22 46%	19 of 22 86%	1 of 22 5%
Internet E-mail Respondents	37	12 of 37 32%	34 of 37 92%	3 of 37 8%
Restaurants	20	20 of 20 100%	12 of 20 60%	0 of 20 0%

^a Fried - crab, if live, is killed. Next, portions to be eaten are removed and placed into a wok where they are fried and mixed with other ingredients.

^b Steamed - live crab placed above boiling water until cooked. Crab opened and portions desired are eaten.

^c Boiled - crab placed in boiling water until cooked. Portions desired are then consumed.

^d Determined from answers indicating if home meals were prepared similarly to those at the restaurant.